

63. (new) A method according to claim 59, wherein the muting nucleic acid comprises a sequence that is homologous to an endogenous sequence located in the 3'-portion of the pro- $\alpha$ 1(I) collagen gene.
64. (new) A method according to claim 63, wherein the 3'-portion of the pro- $\alpha$ 1(I) collagen gene includes a 3' untranscribed portion, a 3' untranslated portion, and a portion that overlaps the 3'-end of the coding portion.
65. (new) A method according to claim 57, wherein delivering the muting nucleic acid further comprises transforming, transfecting, electroporating, infecting, or lipofecting as the means for delivering the nucleic acid into the cells.

#### REMARKS

Preliminarily, Applicant expresses appreciation to Dr. Priebe and Mr. Paras for the courtesy of a telephone interview on February 27, 2002.

#### Claim Objection

The term "two portion" in Claim 17 has been amended to read "two portions" and so the objection by the Examiner has now been addressed.

#### Rejections Under 35 U.S.C. § 112, paragraph one

According to MPEP 2164.01, I(B), to show lack of a enablement under § 112, par. 1, the Examiner is required to establish a *prima facie* case "by providing reasons why a person skilled in the art at the time the invention was filed would not have recognized that the inventor was in possession of the invention .... A general allegation of 'unpredictability in the art' is not sufficient...." [see MPEP 2163.04, I(B)].

The Examiner states that "... the specification, while being enabling for a method of muting expression of a  $\alpha 1(I)$  procollagen in cultured rodent fibroblasts, .... does not reasonably provide enablement for the claimed method comprising any other embodiments." See Office Action, p. 3, main paragraph. The Examiner goes on to say that "In the absence of any relevant teachings with respect to the mechanism of muting, the skilled artisan cannot predict how to achieve muting of other genes. As such, guidance is lacking in the instant specification that teaches the skilled artisan how to construct and use other muting nucleic acid sequences." See Office Action, bottom of p.5 – top of p. 6.

With all due respect, Applicant completely disagrees. As explained in the Specification, to identify a muting nucleic acid composition, one must screen different fragments of DNA from the targeted gene to be muted to determine appropriate sequences of nucleic acid to use as muting sequences. The presently claimed invention requires identification of a nucleic acid muting sequence, no matter how much of the gene is ultimately identified to be required for muting. This is true *even if the muting sequence ultimately determined is the entire gene*. Within that context, the specification provides ample description of how to identify a muting sequence (see p. 4, lines 18-26; or p. 5, lines 26-29 through p. 7, lines 1-8, for example).

In addition, Example 15 explains how one might screen for muting nucleic acids for other genes, for example: 1) the *tat* gene of the human immunodeficiency virus (HIV); 2) the gene encoding tumor necrosis factor alpha (TNF- $\alpha$ ) in vascular endothelial cells; or 3) an immunoglobulin H or L genes in leukocytes. See Specification, pp. 31-33.

Specifically, to screen for muting sequences for the *tat* gene in HIV, the presently claimed invention states that:

Isolation of the smallest effective length of the muting nucleic acid can be achieved by purification and subcloning of different fragments of HIV, starting from within the 5'-LTR (long terminal repeat having the promoter), and extending into the *tat* gene. Initially, large fragments (up to 2 kb) are tested for muting nucleic acid activity. Upon obtaining a positive muting response, the active portion can be isolated by subsequent restriction enzyme digestion, purification of fragments, cloning of each fragment into the vector, and testing each for having a muting activity. See application, p32, lines 18-24.

Note that again, "the smallest effective length" may turn out to be the entire gene. Once muting sequences are identified, the method for muting the corresponding endogenous genes will be analogous to the method detailed for muting the  $\alpha 1(I)$  procollagen gene in rodent fibroblasts.

The Examiner also states that "The specification, however, has not taught how to mute expression of the endogenous  $\alpha 1(I)$  procollagen gene by introducing into cultured animal cells a ribonucleic acid sequence or a nucleic acid analog. The specification has not taught the skilled artisan how to select portions of the  $\alpha 1(I)$  procollagen transcript or a nucleic acid analog which would mute expression...." See Office Action, p. 5. These comments indicate that the Examiner is wrongly assuming that the muting nucleic acid, if RNA or a nucleic acid analog, is selected or screened. The identification of a muting nucleic acid sequence is done *at the level of DNA*, as detailed in the specification (see above). There is no requirement to select portions of an actual  $\alpha 1(I)$  procollagen *transcript*, or to select portions of a nucleic acid analog. Once a muting sequence is identified by screening fragments of DNA, the claimed invention encompasses providing to the cultured population of cells any nucleic acid composition with a sequence

analogous to that identified, whether the composition is DNA, RNA or a nucleic acid analog. This would not require undue experimentation because transfection of RNA and nucleic acid analogs using standard DNA transfection methods is well-known in the art. See, for example, Abstracts 1 and 2, attached as a Supplement with this Response

Throughout the latest Office Action, the Examiner makes assertions and draws conclusions regarding the claimed invention that are not in the application and are wrong, both with respect to the claimed invention, and with respect to the relevant science in general. For example, the Examiner states on p. 7, lines 4-7, that ".... the skilled artisan could interpret the muting effect to be related to the DNA sequence of the exogenous collagen sequences, RNA transcribed from the exogenous collagen sequences, protein produced from the exogenous sequences, ...." As clearly stated in the application, the exogenous collagen sequence is neither transcribed nor expressed as protein. See Application, p.10, lines 13-14 ("Muting of the endogenous gene embodied in the examples herein is independent of expression of the transgene, unlike previously described silencing phenomena.") and p. 13, lines 7-8 (".... i.e., expression of the target endogenous gene was specifically reduced by the presence of the homologous transgene, however the transgene was self-silent ....") and p. 21, lines 22-23 ("Further, expression of the transgenic procollagen gene as determined by synthesis of mRNA was undetectable.")

Similarly, the Examiner comments on p. 8, last par. of the Office Action, that "Even more, with regard to claim 23, if the muting nucleic acid molecule is an RNA molecule it cannot comprise a 3' portion of the gene that is not transcribed. Such a claim embodiment is inoperable because it cannot exist since by definition an RNA molecule is

a transcript of a gene's coding sequences." First, the 3' portion of the gene referred to in claim 23 is the endogenous gene, not the transgene. As amended, claim 23 now makes this clearer.

Second, RNA has come a long way from the limited definition of being only a transcript of a gene's coding sequences. In the claimed invention, there is no requirement that the exogenous gene be transcribed to see muting. Double-stranded RNA, made by annealing *in vitro* run-off transcription can be delivered using standard transfection techniques. Production of RNA run-off transcripts of any desired sequence using T7 RNA polymerase or Sp6 RNA polymerase has been well-known in the relevant art since at least the late 1980s. Such an artificially synthesized and subsequently introduced RNA sequence can easily comprise a sequence that is homologous to an untranscribed portion and a 3' coding portion of the endogenous gene.

In addition, the muting dsRNA sequence may arise from within the cell itself, but not from transcription of the exogenous gene. Rather, its production is merely triggered by delivery of the exogenous DNA muting sequence. In this scenario, the muting dsRNA comes from the *endogenous* gene, but it is not made until the exogenous DNA muting sequence is introduced.

Finally, with respect to the Examiner's objections to RNA or nucleic acid analog compositions as the muting sequence composition, claim 14 has been amended to comprise muting nucleic acids of DNA and DNA analogs only, thereby addressing the Examiner's comments regarding lack of enablement for inserting RNA into a vector. One skilled in the art would understand that DNA analogs can be synthesized and ligated

into a vector, for transfection into a cell using standard techniques. See Abstract 3 in the Supplement for an example of such technology.

With respect to the Examiner's comments regarding the need for undue experimentation to identify muting sequences for other genes (see Office Action, bottom of p. 6 to the top of p.7 – "Without knowledge of the specific sequence(s) responsible for muting, the skilled artisan is not able to predict..."), and the Examiner's opinion that undue experimentation would be required to use RNA as the muting nucleic acid, Applicant respectfully re-asserts that the specification provides more than enough guidance for how to identify such sequences (see Example 15, pp. 31-33 and discussion above). The muting effect is gene specific. The knowledge of the specific sequences responsible for muting other genes comes from screening gene fragments of other genes to identify muting sequences. As stated above and explained in the application, one must identify muting sequences for each gene.

In addition, the Examiner's conclusion that because the effect seems to be sequence specific it cannot be used with other genes is wrong. The sequence specificity merely emphasizes the need to screen for muting sequences, a required element of the claimed invention, and an element the Examiner consistently seems to either not understand, or to forget.

As for the use of RNA, as already discussed above, transfecting cells with run-off RNA transcripts is well-known technology. Furthermore, the delivery of such dsRNA is just into the cell (i.e. the cytoplasm). There is no requirement in the presently claimed invention that the RNA enter the nucleus – that is a requirement imposed by the Examiner. At present, the data suggests that it is the dsRNA which communicates

between the DNA in the nucleus and mRNA in the cytoplasm to effect post transcriptional muting. Knowing the fate of the dsRNA once it gets into the cell, however, is not necessary to realize that it is important and appropriate to claim RNA as a possible muting nucleic acid. At the time of its discovery, RNAi technology completely baffled the scientific world, and in fact still does. Yet it works. And as the Examiner himself pointed out, "The mechanism by which a process occurs does not need to be known for patentability." (See Office Action, p. 6). It is not important that the Examiner himself does not understand how RNA might work as a muting nucleic acid. The fact that RNAi effectively interferes with gene expression in a number of cell systems without the mechanism being known suggests that those skilled in the art will instantly recognize the potential for RNA to be effective in this muting system also, regardless of the mechanism.

Regarding the use of attenuated bacteria as vectors to deliver the muting sequences, the Examiner states on p. 6 that the "specification has not taught how to use attenuated bacteria as a vector." Further, the Examiner discusses on p. 9, last two paragraphs and continuing through all of p. 10, that "The state of the art suggests that use of attenuated bacteria as a means of gene transfer is inefficient, undeveloped and unpredictable..." (see p. 9, second par.) Grillot-Courvalin and Dietrich have been cited to support this position. However, although Grillot-Courvalin starts with the premise that direct introduction of DNA from bacteria to mammalian cells has been reported in very few instances, (see Examiner's quote, top of p. 10 of the Office Action), the article states *in the very next paragraph* that their results "... show that *inv*<sup>+</sup> *E. coli* can deliver DNA at high efficiency into a variety of mammalian cells." (Grillot-Courvalin, p. 865, second

par. of Discussion, emphasis added). As such, Grillot-Courvalin actually provides support of enablement for the claimed invention.

Similarly, like Grillot-Courvalin, Dietrich also provides support that the claimed invention is enabled for using attenuated bacteria or virus to deliver the muting nucleic acid. Again, the Examiner incorrectly interprets the results of Dietrich et al. The Examiner cites Dietrich et al. as claiming only about 0.3% of the delivered genes were expressed (see Office Action, p. 10, citing Dietrich at 183, column 2) for evidence that such a method is not efficient for DNA delivery. But the Dietrich abstract specifically states that "*Efficient* expression of the cloned reporter genes .... were achieved after the delivery of eukaryotic expression vectors by the attenuated suicide *L. monocytogenes* strain." In fact, contrary to the Examiner's assertion, Dietrich does not say *only* about 0.03 % of the delivered genes were expressed, Dietrich says that 0.03% were expressed, *as compared to expression of about 0.001% or nothing* using another strain or a control strain (see column 2 of p. 183, emphasis added.) Dietrich goes on to conclude that "Hence, *efficient* delivery of plasmid DNA into the cytosol of host cells by *L. monocytogenes* is stimulated by .....[the presence of the PLY118 gene]" (id.). Therefore, Applicant respectfully submits that in light of what Grillot-Courvalin et al. and Dietrich et al. actually stand for, the claimed invention is enabled for the use of attenuated bacteria and virus for nucleic acid delivery. As such, the Examiner's assertion of no enablement based on the teachings of those references is in error.

In summary, Applicant respectfully submits that the claimed invention is sufficiently enabled and does not require undue experimentation for one having ordinary skill in the relevant art.



Rejections Under 35 U.S.C. § 112, paragraph two

Claim 11 has been amended to delete the phrase "or non-complementary with respect to mRNA associated with the endogenous gene" from element a). This phrase was added by amendment in Response C, to attempt to distinguish the presently claimed invention from anti-sense RNA technology. However, the phrase erroneously describes the present technology and makes no sense generally or particularly with respect to the presently claimed invention. The muting nucleic acid in accordance with the presently claimed invention is double-stranded. The mechanism by which dsRNA may effect muting, although not required for patentability, is explained below in the context of what role dsRNA is playing in the muting process. Deletion of this phrase merely renders claim 11 consistent with the original disclosure and technology of the presently claimed invention.

Claim 11 has also been amended to re-phrase the claim so that it does not claim a negative. Therefore, in place of "under conditions devoid of selection for integration of the nucleic acid into a chromosomal site so that " the claim has been re-worded to say "independent of integration, expression and transcription."

Finally, claim 11 has been amended to include muting as a separate element (c), and to describe that muting as operating "at levels of transcription and post-transcription" as disclosed in the Specification, p. 23, lines 3-4.

Claim 17 has been amended to eliminate the word "coding" in the original phrase "a transcribed coding portion including introns" so that the phrase now reads "a transcribed portion." Applicant appreciates the fine point expressed by the examiner that

introns, while transcribed, are not considered "coding" regions of a gene, and so claim 17 has been amended to correctly reflect this well-recognized principle.

Claim 23 has been amended to more clearly indicate that the muting nucleic acid has sequence homology to a 3'-portion of an endogenous gene, and that it is the 3'-portion of the endogenous gene that is described by the terms "3'-untranscribed portion", "3'-end coding portion", and "3'-untranslated portion." As amended, Applicant respectfully submits that one of ordinary skill in the art would understand how an exogenous nucleic acid composition could have regions of homology to an untranscribed region of the endogenous gene, and to a 3'-end coding portion and 3'-non-coding portion of the endogenous gene.

In accordance with the presently claimed invention, if the muting nucleic acid is an RNA molecule, it can be generated *in vitro* as a run-off transcript with a sequence having homology to an untranscribed sequence in the endogenous gene, and the muting RNA molecule can be introduced into the cultured cell just as a DNA molecule would be, i.e. via  $\text{Ca}^{2+}$  treatment, electroporation, and the like. It is also possible that such dsRNA may arise through transcription of ssRNA from opposite strands of two separate DNA vectors introduced into the cell. One of ordinary skill in the art would be readily familiar with such concepts. *In vitro* generation of RNA run-off transcripts has been standard procedure in laboratories needing specific RNA sequences since at least the late 1980s, and transcription from opposite strands of a DNA vector has been known for at least as long.

Alternatively, the muting RNA sequence may be generated from the endogenous gene, in response to introduction of an exogenous muting sequence of DNA, and it does

not necessarily have to be mRNA. In any case, it is not a requirement of the presently claimed invention that the muting nucleic acid, if it is RNA, be generated by transcription from the DNA of the exogenous gene, or by transcription inside the cultured cell at all.

To reiterate, the presently claimed invention is *independent* of integration, expression, *or transcription* of the transgene. See specification, p. 10, line 14; p. 14, lines 9-18, lines 20-23, and line 29; p. 21, lines 27-29 to p. 22, lines 1-11; and p. 30, Example 14. Therefore, in light of the clarification in the amended claim, and the above arguments, Examiner's comments regarding homology to untranscribed regions are not on point, and such rejections should be removed.

With respect to amended claims 11, 17, and 23, Applicant respectfully submits that no new subject matter has been added, and the amended claims are in condition for allowance with respect to 35 U.S.C. § 112, paragraph two.

#### Rejections Under 35 U.S.C. § 102(b)

As stated in MPEP 706.02, ".... for anticipation under 35 U.S.C. 102, the reference must teach every aspect of the claimed invention either explicitly or impliedly. Any feature not taught directly must be inherently present."

The Examiner cites Guimaracs et al. as anticipating the present invention because Guimaracs teaches isolation and cloning of Sps2 homologs from mice and humans, that such homologs are found in different species, that transfection of a plasmid comprising such homologs was performed in COS 7 cells without disrupting the endogenous Sps2 sequence, and that the sequence of the Sps2 cDNA sequence is homologous to 5' and 3' regions as well as a portion that overlaps adjacent ends. See p. 14 of Office Action, first

full paragraph, generally. However, nowhere in the Guimaraes et al. reference does it say there actually is an endogenous Sps2 gene in the COS-7 cells (monkey kidney cells). The Guimaraes references monitors expression of the exogenous *mouse* Sps2 gene in the COS-7 cells, it does not monitor, or mention the possibility of monitoring, expression of an original endogenous *monkey* Sps2 gene. See p.15087, col. 1, second heading ("**Generation of Sps2 Constructs for Protein Expression in COS-7 Cells.**") and throughout the reference. This is nothing like the presently claimed invention, whereby an endogenous gene is muted by the presence of an exogenous muting sequence, and is therefore not a relevant reference.

Furthermore, the Examiner states: "If the muting method is general in effect to all genes as asserted in the specification then it is inherent that expression of the endogenous Sps2 gene in COS-7 cells must be inhibited. The claim only requires that the muting nucleic acid be homologous to a sequence in the endogenous gene...." *Id.*

First, the claim does **not** "*only* require that the muting nucleic acid be homologous to a sequence in the endogenous gene...." The claims also require a step for "identifying a muting nucleic acid composition having a sequence that is homologous..." See claim 11. The step of identifying the muting nucleic acid composition is not inconsequential. It is not enough to identify a nucleic acid composition homologous to a sequence in the endogenous gene, as the Examiner wrongly assumes, thereby compounding his error by concluding that all sequences with some homology to the endogenous gene fall under the umbrella of the claimed invention. The claims of the present invention require "identifying a *muting* nucleic acid composition...." *Id.* Therefore, any reference cited by the Examiner which does not utilize a nucleic acid fragment *identified to have muting*

*capabilities* is not an appropriate reference, since the presently claimed invention requires identification of a *muting* nucleic acid.

For the Examiner to assert that the claim "only requires that the muting nucleic acid be homologous to a sequence in the endogenous gene" is a fundamental error in logic. The assertion is equivalent to an argument that if certain trees have red leaves, and you have a plant with red leaves, then you must have a tree. Or, if a claim requires identification of a pain-reducing compound having a sugar that binds to a galactose receptor, it does not follow that a compound having a sugar that binds to a galactose receptor will be a pain-reducing compound. The claim at issue here requires homology to be in the identified sequence, but the identified sequence must also be a muting sequence.

Applicant also objects to the Examiner's sweeping comment that "If the muting method is general in effect to all genes *as asserted in the specification* then it is inherent that expression of the endogenous Sps2 gene in COS-7 cells must be inhibited." See Office Action, p. 14, 7<sup>th</sup> line from bottom, emphasis added. The Examiner has provided no support for where in the specification Applicant has made such an assertion, and a thorough search of the specification shows that no such statement exists. What the Applicant *does* say is "The methods and compositions of various embodiments of the present invention can be used to mute an endogenous gene in an animal cell .... or alternatively, can be used to mute a viral gene such as a gene encoding a coat protein from HSV-II or from HIV" (specification, p. 11, lines 27-29 to p. 12, line 1). To state that the *methods* of the presently claimed invention *can* be used to mute genes in an animal cell or a virus is very different from asserting that "the muting effect is general in

effect to all genes" as claimed by the Examiner. The methods of the claimed invention require identification of a muting nucleic acid. The methods of the presently claimed invention do not apply unless there has first been identification of a muting sequence nucleic acid for a particular gene.

As stated in *In re Rijckaert*, 9 F.3d 1531, 1534 (Fed Cir. 1993) (see also MPEP 2112), "The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic." And as reiterated by the Federal Circuit in 1999 regarding rejections based on inherency, "The mere fact that a certain thing may result from a given set of circumstances is not sufficient." See *In re Robertson*, 169 F.3d 743, 745 (Fed. Cir. 1999) (see also MPEP 2112).

As stated above, it is important to recognize that the muting nucleic acid molecules, whether DNA, RNA, or nucleic acid analogs, effectuate muting of the *endogenous* gene *independent* of integration, expression, or transcription of the *exogenous* nucleic acid molecule introduced. Further, the claimed methods require identification of a muting nucleic acid before the muting effect can be seen. The limitations that such requirements place on the claimed invention cannot be stressed enough.

Guimaraes, et al, Chan et al., Rippe et al., and Slack et al., are not relevant as references because none identifies a muting nucleic acid composition for a targeted endogenous gene, and none investigates muting of an endogenous gene. Guimaraes et al. looked at expression of a mouse Sps2 gene in monkey COS-7 cells where the mouse Sps2 gene was present on a pME18X vector that was transfected into the COS-7 cells.

The entire reference relates to successful transfection and *expression* of the *exogenous* DNA introduced into the COS-7 cells. The Guimaraes reference does not identify a muting nucleic acid sequence to a targeted gene, and it does not discuss muting of any gene, endogenous or exogenous.

Similarly, Chan et al. involves insertion of a retroviral provirus into the first intron of the murine  $\alpha 1$  type I collagen gene (COL1A1) and its effect on expression of the COL1A1 gene via changes in DNA methylation. The Chan reference does not teach identification of a muting sequence of nucleic acid, and no muting sequence was identified and subsequently delivered to a cultured cell in the Chan reference. The effect of an *inserted and expressed* retroviral provirus on expression of an endogenous gene is not at all relevant to the presently claimed invention. The muting method of the claimed invention requires: a) identification of a muting nucleic acid, b) delivery to a cell, and 3) muting of a targeted endogenous gene wherein the muting is *independent of integration, expression, or transcription* of the delivered exogenous nucleic acid.

Rippe et al. is relevant only as support for the claimed invention to show how one might identify and select gene sequences that could be effective as muting sequences for a particular gene, in this case the mouse alpha 1 type collagen gene (COL1A1). However, Rippe et al. only teaches identification of regulatory sequences that effect expression of an exogenous fusion protein containing portions of the COL1A1 gene (see p. 2224, col. 1, first paragraph, and legend to Fig. 1). There is no teaching of using such identified sequences as exogenous muting nucleic acid which, when introduced into a cell, can effect muting of the *endogenous* gene, as defined in the presently claimed invention.

Slack et al. teaches the effect of the oncogene *ras* on expression of type I collagen in rodent fibroblasts. The Slack reference teaches the use of cloned Rat 1 fibroblasts containing the MMTV-N-*ras*<sup>Lys 61</sup> plasmid, wherein the *expression* of the oncogenic *ras* protein is what effects expression of endogenous type I collagen. See Slack, p. 4716, **Results** section. Slack et al. does not teach identification of an exogenous muting nucleic acid composition, capable of muting the *endogenous* gene of interest *independent* of integration, *expression*, or *transcription* of the *exogenous* nucleic acid.

The Examiner argues that because each of the references introduces exogenous nucleic acids into a cell, and those nucleic acids have regions of sequence homology to an endogenous gene, the presently claimed invention is anticipated because muting is inherent in homologous sequences. As discussed more fully above, just because something is possible does not make it inherent. In addition, the claimed invention requires *identification* of a *muting* nucleic acid as an element of the method for muting. After identification of a muting sequence, there must also be delivery of the identified muting sequence to a population of cultured cells such that, *independent* of integration, expression, or transcription of the muting nucleic acid, muting of the endogenous gene occurs.

And finally, Gambarotta et al., teaches use of double-stranded oligonucleotides having *Ets* consensus sequence to inhibit binding of *Ets* transcription factors to the promoter of *MET*, thereby reducing the amount of *MET* protein present in a cell line overexpressing the oncogene *MET* (see Gambarotta, p. 1911, Abstract).

Basically, Gambarotta et al. identified regions (*Ets* transcription factor binding sites) in the oncogene *MET* responsible for up-regulation of the promoter for the *MET*



gene. The binding sites were verified by showing that co-expression of *Ets1* resulted in strong enhancement of the MET promoter activity. *Id.* By adding the inhibitor oligonucleotides, binding of the *Ets* transcription factors to the promoter of the oncogene *MET* was prevented because the transcription factors bound to the oligonucleotide inhibitors instead, thereby preventing over-expression of the *MET* protein. *Id.*, pp. 1913-1914. In effect, the authors designed a classic competitive inhibitor of the *Ets* transcription factors, reducing gene expression at the level of transcription.

In contrast, the presently claimed invention does not occur through reduction of endogenous gene expression at the level of transcription alone. Rather, muting of the presently claimed invention occurs through a combination of transcriptional and post-transcriptional effects. See specification, p. 14, lines 24-27 ("The 3' portion of the  $\alpha 1(I)$  procollagen gene present in pWTC1 carries some additional regulatory elements which effect post-transcriptional muting..."); p. 22, lines 20-23 ("Evidence exists for degradation of the pre-transfection population of the procollagen mRNA shortly after ectopic transfection by pWTC1...."); p. 23, lines 3-4 ("Thus the gene muting observed here was found to be partly due to a post-transcriptional component."); and p. 30, lines 15-18(".... the regulatory element(s) present at the 3' region of this gene .... effect post-transcriptional muting of this gene.").

Gambaretta et al teaches a classic competitive inhibitor wherein at high concentration of inhibitor, saturation binding of the transcriptional factors occurs, and maximum inhibitory effect is seen through inhibition of transcription alone. See Gambaretta, et al., p. 1914, Fig. 6. The present invention discloses muting of an

endogenous gene through a combination of both transcriptional and post-transcriptional events.

In addition, the "decoy" oligonucleotides used for reducing expression of Met gene were not incorporated into a plasmid, as suggested by the Examiner. They were electroporated into the cells as free double-stranded oligonucleotides. The ds-oligonucleotides are 11 base-pairs and comprise a tetra-nucleotide (GGAA) sequence that is recognized by members of the Ets family. GGAA, statistically speaking, is a commonly occurring sequence and is present by thousands throughout the genome. In a 690-bp DNA fragment shown in Figure 2 of Gambaretta, this sequence is found six times. Thus, the inhibition of expression is not gene specific. In fact, in Figure 6, Gambaretta et al. show that expression of another gene, receptor protein p185HER2, was also decreased by the same oligonucleotides.

In contrast, the present invention as claimed relies on the insight that the expression of an endogenous gene in a cell population can be efficiently and specifically muted by coordinated transcriptional and post-transcriptional mechanisms induced by exogenous copies of the endogenous gene. Therefore, Gambaretta does not disclose the third element of the claimed invention (see claim 11, element (c), above).

To summarize, the sequence elements relevant to the muting mechanisms are gene- (or gene family-) specific, and seem to be polarized; transcriptional elements present at the 5' end and post-transcriptional elements at the 3' end of the gene. They are separable, as shown for the procollagen gene example. However, the entire transcribed portion, as well as some untranscribed portions of a gene, could be relevant for efficient and specific muting of some genes. The presently claimed invention does not operate to effect gene muting by simple competitive inhibition of transcription factors to the

promoter regions of the gene. The presently claimed invention operates through a combined transcriptional and post-transcriptional process. *Vide supra* p. 20. The data suggests that it is dsRNA that most likely signals the degradation of mRNA from the endogenous gene in the post-transcriptional mechanism. See Specification, p. 23, lines 3-4.

To one with ordinary skill in the art, it would be understood that the intermediate molecule for transmission of signals from DNA in the nucleus to mRNA in the cytoplasm must be RNA. As would be understood by one with ordinary skill in the art, this RNA triggers the degradation of specific mRNA during post-transcriptional muting. Because mRNA cannot signal its own degradation, the triggering RNA is most likely double-stranded RNA. As such, in the presently claimed muting processes, DNA analogs of muting DNA are expected to act like identified muting DNA to signal production of dsRNA from the endogenous gene, and analogs of RNA are expected to substitute for double-stranded RNA to trigger degradation of mRNA from the endogenous gene, thereby effecting muting.

Further, fragments of a gene from either end could bring about partial muting, as opposed to the most efficient type of muting by the entire gene sequence. Knowledge of the precise sequence of the muting elements is not necessary to practice the technology of the presently claimed invention. Knowledge of the approximate location of these elements, identified, for example, by restriction mapping, is sufficient if the complete gene sequence is not to be used.

Relative to the prior art cited against the presently claimed invention, not one teaches a) identification of a muting sequence, b) delivery of the muting sequence, and (c) muting of a desired endogenous gene at the levels of transcription and post

transcription, independent of integration, expression, or transcription of said muting nucleic acid.

None of the prior art cited against this application is relevant, because none teaches identification of a muting nucleic acid sequence which, when introduced into a cultured cell, is capable of effecting muting of a desired endogenous gene, independent of integration, expression, or transcription of the exogenous nucleic acid. As such, there is no anticipation by the cited reference of Applicant's claimed invention.

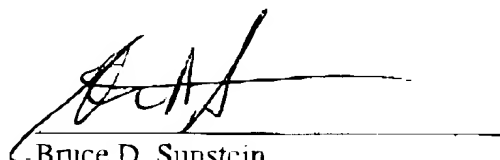
It is therefore respectfully submitted that all pending claims are in condition for allowance. Reconsideration of the claims, consideration of the added claims, and a notice of allowance is therefore requested.

You are hereby authorized to charge deposit account number 19-4972 the fee of \$55,000 to cover the fee for a one-month extension of time. It is believed that no additional extension is needed; however, this conditional petition for an additional extension of time is being made in the event that the need for more than a one-month extension has been overlooked. If any additional fees are required for the timely consideration of this application, please charge deposit account number 19-4972. The Examiner is requested to telephone the undersigned if any matters remain outstanding so that they may be resolved expeditiously.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment, captioned "Version With Markings to Show Changes Made."

Date: May 30, 2002

Respectfully submitted,

A handwritten signature in dark ink, appearing to read "B. Sunstein", is written over a horizontal line.

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Version with Markings to Show Changes

11. (thrice amended) A method for muting expression of an endogenous gene in a cultured population of animal cells, the method comprising the steps of:

(a) identifying a muting nucleic acid composition having a sequence that is homologous to a sequence in the endogenous gene, the nucleic acid composition being double stranded [~~or non-complementary with respect to mRNA associated with the endogenous gene~~]; and

(b) delivering the muting nucleic acid into the population of cells [~~under conditions devoid of selection for integration of the nucleic acid into a chromosomal site so that~~]; and

(c) muting expression of the endogenous gene at levels of transcription and post-transcription in the population as a whole [~~is inhibited~~], wherein such muting is independent of integration, expression, or transcription of the delivered nucleic acid [~~even though such gene's sequence is not therein disrupted~~].

14. (once amended) A method according to claim [13] 11, wherein [(a) further comprises] the nucleic acid is DNA or analogs of DNA, further comprising the step of engineering the [nucleic acid] DNA or analog of DNA into a recombinant vector before the delivering step.

17. (Thrice amended) A method according to claim 11, wherein the muting [~~transgene sequence~~] nucleic acid composition is homologous to an endogenous sequence comprising a portion of the endogenous gene selected from at least one of the group of: a 5' untranscribed portion, a transcribed [~~coding~~] portion [~~including introns~~], a 3'

untranslated portion, a 3' untranscribed portion, and a portion that overlaps adjacent ends of at least two ~~[portion]~~ portions of the endogenous gene.

23. (Once Amended) A method according to claim 22, wherein ~~[the 3' portion of the gene includes an]~~ the muting nucleic acid comprises a sequence that is homologous to an endogenous sequence located at the 3'-portion of the gene, said endogenous sequence including a 3' untranscribed portion, a 3' untranslated portion, and a [portion that overlaps the] 3' end [of the] coding portion.

57. (new) A method for muting expression of an endogenous gene in a cultured population of animal cells, the method comprising:

(a) identifying a muting nucleic acid composition having a sequence that is homologous to a sequence in the endogenous gene, wherein the gene is one of a collagen, tumor necrosis factor (TNF), *tat*, and an immunoglobulin gene, the nucleic acid being double stranded; and

(b) delivering the muting nucleic acid into the population of cells; and

(c) muting expression of the endogenous gene at levels of transcription and post-transcription in the population as a whole, wherein such muting is independent of integration, expression, or transcription of the delivered nucleic acid.

58. (new) A method according to claim 57, wherein the endogenous gene is a type I collagen.

59. (new) A method according to claim 58, wherein the endogenous gene is pro- $\alpha 1(I)$  collagen.

60. (new) A method according to claim 57, wherein the cultured population of animals are rodent cells.

61. (new) A method according to claim 59, wherein the muting transgene sequence is homologous to an endogenous sequence comprising a portion of the pro- $\alpha 1(I)$  collagen gene selected from at least one of the group of: a 5'-untranscribed portion, a transcribed portion, a 3'-untranslated portion, a 3'-untranscribed portion, and a portion that overlaps adjacent ends of at least two portions of the pro- $\alpha 1(I)$  collagen gene.

62. (new) A method according to claim 59, wherein the muting nucleic acid comprises a sequence homologous to an endogenous sequence located in the 5'-portion of the pro- $\alpha 1(I)$  collagen gene.

63. (new) A method according to claim 59, wherein the muting nucleic acid comprises a sequence that is homologous to an endogenous sequence located in the 3'-portion of the pro- $\alpha 1(I)$  collagen gene.

64. (new) A method according to claim 63, wherein the 3'-portion of the pro- $\alpha 1(I)$  collagen gene includes a 3' untranscribed portion, a 3' untranslated portion, and a portion that overlaps the 3'-end of the coding portion.

65. (new) A method according to claim 57, wherein delivering the muting nucleic acid further comprises transforming, transfecting, electroporating, infecting, or lipofecting as the means for delivering the nucleic acid into the cells.



# SUPPLEMENT

- Abstract 1**      **“PNA-dependent gene chemistry: Stable coupling of peptides and oligonucleotides to plasmid DNA”**
- Abstract 2**      **“Antisense oligodeoxynucleotide targeted to midkine, a heparin-binding growth factor, suppress Tumorigenicity of mouse rectal carcinoma cells.**
- Abstract 3**      **“RNA-Mediated RNA Degradation and Chalcone Synthase A Silencing Petunia”**

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# Abstract # 2

Biological Abstracts 1980 - Present  
Full Citation

You searched for (TRANSFECTION.SU.) AND PNA. This is record 3 out of 4.

Author Zelphati, O.; Liang, X.; Nguyen, C.; Barlow, S.; Sheng, S.; Shao, Z.; Felgner, P. L.

Author Address Gene Therapy Systems, 10190 Telesis Court, San Diego, CA, 92121, USA.

Title PNA-dependent gene chemistry: Stable coupling of peptides and oligonucleotides to plasmid DNA.

Appears In *Biotechniques* 28(2) Feb 2000. 304-316.

Abstract Two approaches are described for stably conjugating peptides, proteins and oligonucleotides onto plasmid DNA. Both methods use a peptide nucleic acid (PNA) clamp, which binds irreversibly and specifically to a binding site cloned into the plasmid. The first approach uses a biotin-conjugated PNA clamp that can be used to introduce functional biotin groups onto the plasmid to which streptavidin can bind. Atomic force microscopy images of linearized plasmid show streptavidin localized at the predicted PNA binding site on the DNA strand. Peptides and oligonucleotides containing free thiol groups were conjugated to maleimide streptavidin, and these streptavidin conjugates were bound to the biotin-PNA-labeled plasmid. In this way, peptides and oligonucleotides could be brought into stable association with the plasmid. A second approach used a maleimide-conjugated PNA clamp. Methods are described for conjugating thiolated peptides and oligonucleotides directly to the maleimide-PNA-DNA hybrid. This straightforward technology offers an easy approach to introduce functional groups onto plasmid DNA without disturbing its transcriptional activity.

Concept Codes \* Biochemical Methods-Nucleic Acids, Purines and Pyrimidines  
\* Biochemical Studies-Proteins, Peptides and Amino Acids \* Biophysics-General Biophysical Techniques \* Genetics of Bacteria and Viruses

Major Concepts Molecular Genetics (Biochemistry and Molecular Biophysics). Methods and Techniques.

Chemicals oligonucleotides; peptide nucleic acid; peptides; plasmid DNA.

Methods and Equipment AITTO-TAG labeling kit: Molecular Probes, equipment. agarose gel electrophoresis: analytical method, gel electrophoresis. atomic force microscopy: microscopy method, microscopy: CB, microscopy: CT. transfection: gene expression/vector techniques, genetic method. transmission electron microscopy: electron microscopy: CB, electron microscopy: CT, microscopy method.

Descriptors PNA-dependent gene chemistry.

Publication Type Article

Language English

ISSN 0736-6205

Accession BACD200000183099  
Number

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Abstract # 2

Biological Abstracts 1980 - Present  
Full Citation

You searched for (TRANSFECTION.SU.) AND PHOSPHOROTHIOATE. This is record 2 out of 21.

Author Takei, Yoshifumi; Kadomatsu, Kenji; Matsuo, Seiichi; Itoh, Hiroshi; Nakazawa, Kunihiko; Kubota, Shunichiro; Muramatsu, Takashi

Author Address Department of Biochemistry, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, 466-8550, Japan  
Department of Biochemistry, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, 466-8550, Japan  
E-Mail: tmurama@med.nagoya-u.ac.jp.

Title Antisense oligodeoxynucleotide targeted to midkine, a heparin-binding growth factor, suppresses tumorigenicity of mouse rectal carcinoma cells.

Appears In *Cancer Research* 61(23) December 1 2001. 8486-8491.

Abstract Midkine (MK), a heparin-binding growth factor, is overexpressed in a wide range of human carcinomas and is believed to contribute to tumorigenesis and tumor progression. To develop an antitumor reagent, we designed a phosphorothioate antisense oligodeoxynucleotide molecule based on the secondary structure of MK mRNA. The antisense MK at the dosage of 5  $\mu$ M suppressed MK production by CMT-93 mouse rectal carcinoma cells after cationic liposome-mediated transfection, to 13% of that in control cultures. The growth of CMT-93 cells and their colony formation in soft agar were inhibited by the addition of the antisense MK, whereas the control reagent, the sense MK, showed no effects. On s.c. injection into nude mice, CMT-93 cells transfected with the antisense MK formed tumors much smaller than those by control cells. Finally, untreated CMT-93 cells were inoculated to nude mice, and 7 days later the antisense MK (50  $\mu$ M) with atelocollagen was directly injected into the preformed tumor region to evaluate the curative effect; the injection was repeated at the interval of 2 weeks. During the period of 10-41 days after initiation of therapy, the rate of increase of tumor volume treated with the antisense MK was found to be about 4.2-fold lower than that seen after treatment with the sense MK. On this occasion, proliferation of tumor cells as estimated by 5-bromodeoxyuridine incorporation was strongly inhibited, whereas angiogenesis was less affected. These findings strongly suggested the usefulness of MK antisense oligodeoxynucleotide as a new reagent for cancer therapy.

Concept Codes \* Cytology and Cytochemistry-Animal \* Biochemical Studies-Carbohydrates \* Digestive System-Physiology and Biochemistry

\* Digestive System-Pathology \* Neoplasms and Neoplastic Agents-Pathology; Clinical Aspects; Systemic Effects

Major Concepts Digestive System (Ingestion and Assimilation). Tumor Biology.

Biosystematic 86375.  
Code

Taxonomic Muridae.  
Categories

Organisms Muridae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia. CMT-93 cell line (Muridae): mouse rectal carcinoma cells; mouse (Muridae). Animals; Chordates; Mammals; Nonhuman Mammals; Nonhuman Vertebrates; Rodents; Vertebrates..

Chemicals 5-bromodeoxyuridine; atelocollagen; heparin; midkine: heparin-binding growth factor; midkine messenger RNA; phosphorothioate midkine antisense oligonucleotide. 59-14-3: 5-BROMODEOXYURIDINE; 9005-49-6: HEPARIN.

Parts & Structures rectal carcinoma cells: digestive system, pathological structure, tumorigenicity. rectum: digestive system.

Diseases rectal carcinoma: digestive system disease, neoplastic disease.

Methods and Equipment cationic liposome-mediated transfection: cytogenetic method.

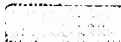
Descriptors tumor cell proliferation.

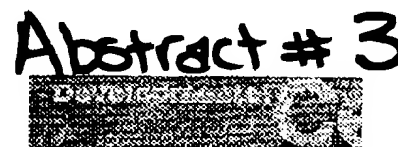
Publication Type Article

Language English

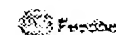
ISSN 0008-5472

Accession BACD200200019532  
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Cell 88: 845.

### Summary 1 of 1

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Cell, Vol. 88, 845-854, March, 1997

## RNA-Mediated RNA Degradation and Chalcone Synthase A Silencing Petunia

M. Metzloff<sup>1</sup>, M. O'Dell<sup>1</sup>, P. D. Cluster<sup>1</sup>, and R. B. Flavell<sup>1</sup>

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Transgenic Petunia plants with a *chsA* coding sequence under the control of a 35S promoter sometimes lose endogenous and transgene chalcone synthase activity and purple flower pigment through posttranscriptional *chsA* RNA degradation. In these plants, shorter poly(A)<sup>+</sup> and poly(A)<sup>-</sup> *chsA* RNAs are found, and a 3' end-specific RNA fragment from the endogene is more resistant to degradation. The termini of this RNA fragment are located in a region of complementarity between the *chsA* 3' coding region and its 3' untranslated region. Equivalent *chsA* RNA fragments remain in the white flower tissue of a nontransgenic Petunia variety. We present a model involving cycles of RNA-RNA pairing between complementary sequences followed by endonucleolytic RNA cleavages to describe how RNA degradation is likely to be promoted.

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